

Patent Application of

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for

(-)-HYDROXYCITRIC ACID FOR DELAYING GASTRIC EMPTYING

BACKGROUND OF THE INVENTION

1. Field Of The Invention

The invention relates to the use of food and pharmaceutical compositions containing (-)-hydroxycitric acid, its salts, amides and esters for influencing glucagon-like peptides (GLP-1/2) and cholecystokinin (CCK), delaying gastric emptying and increasing gastric receptive relaxation for preventing and treating diverse conditions.

2. Description Of Prior Art

Altered gastric emptying and accommodation characterize a number of disease conditions.

Gastric accommodation to distension from an influx of food, also called receptive relaxation, can prevent the change in total intragastric pressure despite an increase in stomach contents. Altered rates of gastric emptying often are accompanied by various health problems with the wall of the stomach itself or issues involving neighboring organs. Thus there appear to be links from altered gastric emptying rates to conditions as seemingly diverse as stomach ulcers and portal hypertension as well as more to be expected conditions, such diabetes and obesity.

That diverse conditions are linked to altered rates of gastric emptying reflects the fact that gastric motility is controlled, at least in part, by vagal inhibitory neurons, various postganglionic nerves and a variety of endocrine and non-endocrine compounds. Among the proposed compounds are acetylcholine, norepinephrine, secretin, glucagon, motilin, glucagon-like peptides,

peptide YY and serotonin. Unfortunately, the evidence for most of these remains surprisingly inconsistent and controversial. Hence, proposing mechanisms often is remote from demonstrating practical methods for delaying gastric emptying. For instance, although serotonin is produced and released by a number of gastrointestinal neurons, the use of compounds that powerfully influence serotonin reuptake or otherwise act as agonists in clinical experiments has failed to significantly affect gastric emptying.

Accelerated gastric emptying and a reduction of gastric accommodation are symptoms often found in hypertension caused by an obstacle to portal blood circulation. Blockages of this sort provoke congestion of the stomach wall and the intestine as well as functional disorders in these viscera. (Aprile LR, Meneghelli UG, Martinelli AL, Monteiro CR. Gastric motility in patients with presinusoidal portal hypertension. *Am J Gastroenterol*. 2002 Dec;97(12):3038-44.) Gastric emptying in liver cirrhosis may similarly be accelerated. This symptom in cirrhosis is primarily found with smaller and more liquid meals, which is unfortunate because the emptying of larger meals in these patients, which tends to be either more or less normal or even delayed, remains improperly coordinated with bile release, which is, again, inadequate. (Acalovschi M, Dumitrascu DL, Csakany I. Gastric and gall bladder emptying of a mixed meal are not coordinated in liver cirrhosis--a simultaneous sonographic study. *Gut*. 1997 Mar;40(3):412-7.)

Ulcers constitute another set of conditions that are characterized by dysregulations in gastric emptying. Gastric emptying is rapid in patients with proximal gastric ulcer due to accelerated proximal evacuation. Similarly, rapid emptying is seen in duodenal ulcer patients and is considered to be due to accelerated emptying in both the proximal stomach and the antrum. However, emptying is delayed in patients with distal gastric ulcer due to reduced emptying in the antrum. Gastric emptying in the healing stage is closer to that found in healthy subjects than in patients with active-stage ulcer.

Of common ulcers, duodenal ulcers most likely would benefit from delaying gastric emptying and a reduction in the excessive stomach acid entering the duodenum. Recent research bears this out and indicates that drug-induced ulcers and non-*H. pylori* ulcers may be more common than once thought. "It is increasingly recognized that different causes of ulcers coexist in a given patient, confounding determination of the exact cause of the ulcer. For example, in infected patients with ulcers who also are using nonsteroidal anti-inflammatory drugs (NSAIDs), it is not

possible to establish the ulcer's cause. Moreover, recent studies in the United States in infected patients with duodenal ulcers who were treated with various regimens to prove their efficacy in eradicating *H. pylori* and preventing ulcer recurrence found that approximately 20% of patients suffered an ulcer recurrence despite successful *H. pylori* eradication. The infection clearly did not cause their ulcers but was originally thought to have done so. Thus, as many as one-fifth of patients with ulcers may have the cause falsely attributed to *H. pylori* infection. When this number is added to that of ulcer patients who are *H. pylori*-negative upon original presentation--at least 20% in other recent U.S. studies--it is evident that the proportion of non-*H. pylori* ulcer patients is larger than originally believed. This proportion is likely to increase with the declining incidence of *H. pylori* infection. Other causes of ulcers include the use of aspirin and NSAIDs (which may be surreptitious), hypersecretory states, Crohn's disease, and patients with "idiopathic" ulcers. Patients with "idiopathic" ulcers are characterized by postprandial hypersecretion of acid and hypergastrinemia with accelerated gastric emptying." (Freston JW. *Helicobacter pylori*-negative peptic ulcers: frequency and implications for management. J Gastroenterol. 2000;35 Suppl 12:29-32.)

Among the possible contributory causes of ulcers are recent diet drugs. Orlistat in particular has been shown to speed gastric emptying while at the same time increasing postprandial gastric acidity. This is the pattern already noted in duodenal ulcers. Inasmuch as lipase release plays an important role in reducing gastric acidity and in inhibiting gastric emptying (Borovicka J, et al. Role of lipase in the regulation of postprandial gastric acid secretion and emptying of fat in humans: a study with orlistat, a highly specific lipase inhibitor. Gut. 2000 Jun;46(6):774-81), it is likely that other lipase inhibitors, as well, may contribute to seldom recognized side effects, such as challenges to the integrity of the duodenum.

In contrast with Orlistat, at least one item used for weight loss actually protects against ulcer formation. *Garcinia cambogia* extract has been tested for its anti-ulcerogenic effect. Oral pretreatment of rats with *Garcinia cambogia* fruit extract (1 g/kg body wt/day) for 5, 10 or 15 days protected the gastric mucosa against the damage induced by indomethacin (20 mg/kg body wt). The volume and acidity of the gastric juice decreased in the pretreated animals. The glycoprotein levels of the gastric contents were decreased in the untreated rats, but remained at near normal levels in the pretreated animals. Likewise, protein was elevated in the gastric juice of

untreated rats but, again, remained near normal levels in the pretreated rats. The extract was able to decrease the acidity and to increase the mucosal defence in the gastric areas. (Mahendran P, Vanisree AJ, Shyamala Devi CS. The antiulcer activity of *Garcinia cambogia* extract against indomethacin-induced gastric ulcer in rats. *Phytother Res.* 2002 Feb;16(1):80-3.) Similar protective effects have been reported against alcohol-induced ulceration. (Mahendran P, Sabitha KE, Devi CS. Prevention of HCl-ethanol induced gastric mucosal injury in rats by *Garcinia cambogia* extract and its possible mechanism of action. *Indian J Exp Biol.* 2002 Jan;40(1):58-62.)

As can be seen from the foregoing, accelerated gastric emptying is associated with a variety of medical conditions. Altered gastric emptying and accommodation are found with forms of hypertension, liver dysfunction and gastrointestinal ulcers. Numerous medications, such as antibiotics (erythromycin, indomethacin, etc.) and including even some diet drugs, can accelerate gastric emptying. Surgery, such as for peptic ulcers, itself can lead to clinical dumping syndrome, as can other types of surgery performed on the stomach. A recent specialty publication gave its own list as follows: “The factors or conditions that lead to normal acceleration of gastric emptying include coffee, cigarette smoking, obesity, high-energy density of food, fat intolerance, and hypertension. The conditions that can lead to abnormal acceleration of gastric emptying and symptoms mimicking EDS include idiopathic etiology, subtotal gastrectomy, early stages of noninsulin-dependent diabetes mellitus, Zollinger-Ellison syndrome, and duodenal ulcer.” (Singh A, Gull H, Singh RJ. Clinical significance of rapid (accelerated) gastric emptying. *Clin Nucl Med.* 2003 Aug;28(8):658-62.)

The inventors have made the quite surprising discovery of a family of compounds that can be used to delay gastric emptying. This discovery is particularly surprising in that these compounds have been rigorously studied for over forty years. We have found that (–)-hydroxycitric acid, its salts, amides and esters are useful for delaying gastric emptying and for increasing receptive relaxation and thus can be utilized for preventing and/or treating diverse conditions. We first mentioned this effect on gastric emptying in a provisional patent filing on 14 September 1999 (PPA No. 60/153841), but were not at that time in a position to follow up on our invention.

(–)-Hydroxycitric acid (abbreviated herein as HCA) a naturally-occurring substance found

chiefly in fruits of the species of *Garcinia*, and several synthetic derivatives of citric acid have been investigated extensively with regard to their ability to inhibit the production of fatty acids from carbohydrates, to suppress appetite, and to inhibit weight gain. (Sullivan C, Triscari J. Metabolic regulation as a control for lipid disorders. I. Influence of (–)-hydroxycitrate on experimentally induced obesity in the rodent. *Am J Clin Nutr.* 1977 May;30(5):767-76.) Numerous other benefits have been attributed to the use of HCA, including, but not limited to an increase in the metabolism of fat stores for energy and an increase in thermogenesis (the metabolism of energy sources to produce body heat in an otherwise wasteful cycle). The commonly offered explanation for the effects of HCA is that this compound inhibits the actions of cytoplasmic (cytosolic) ATP:citrate lyase. (Cloutre D, Rosenbaum ME. *The Diet and Health Benefits of HCA (Hydroxicitric Acid)*, 1994.) Weight loss benefits were attributed to HCA, its salts and its lactone in United States Patent 3,764,692 granted to John M. Lowenstein in 1973. Lowenstein described a variety of possible pharmaceutical salts of HCA based upon alkali metals, e.g., potassium and sodium, and alkaline earth metals, e.g., calcium and magnesium.

Free (–)-hydroxycitric acid, calcium, magnesium and potassium salts of HCA and poorly characterized mixtures of two or more of these minerals, usually substantially contaminated with sodium, currently exist on the American market. Calcium/sodium salts have been sold widely since at least as early as 1992. Most of the HCA sold to date consists of calcium salts of varying degrees of purity and, more recently, of poorly characterized calcium and potassium mixtures.

HCA was very extensively studied by Hoffman-La Roche. The researchers over a period of years consistently maintained in numerous peer-reviewed journal articles and elsewhere that HCA does not influence gastric emptying. This position was borne of the conviction that all of the appetite-suppressing effects of the compound arise from its impact upon the liver and the activation of sugar-sensing neurons. Tests to establish the appetite suppressing effects of HCA found that a single large oral dose or two divided oral doses totaling approximately one-fourth the size of the single dose resulted in a 10% or greater reduction in food consumption in experimental animals fed a high-sugar diet. (Minimum doses were 2.63 mmoles/kg once per day or 0.33 mmoles/kg twice per day either one hour before meals or four hours after, but not after the last meal of the day.) This result continued over many weeks with the chronic ingestion of HCA. The appetite control mechanism of HCA was said to not involve any conditioned aversion to

food, i.e., HCA does not alter taste, cause gastric distress or illness, etc. Rather, this control was thought to stem from the increased production of glycogen and/or stimulation of glucoreceptors in the liver, either of which results in early satiety through signals sent to the brain via the vagus nerve. In at least eight different publications ranging in years from 1976 to 1985, Roche researcher Ann C. Sullivan and co-workers argued that HCA does not affect gastric emptying. It should be noted that researchers Sullivan and Triscari were aware at least as early as 1976 that duodenal glucose receptors regulate appetite, yet they never made the connection with HCA.

Sullivan C, Triscari J. Possible interrelationship between metabolite flux and appetite. In D. Novin, W. Wyriwicka and G. Bray, eds., *Hunger: Basic Mechanisms and Clinical Implications* (New York: Raven Press, 1976) 115-125.

Sullivan C, Triscari J. Metabolic regulation as a control for lipid disorders. I. Influence of (–)-hydroxycitrate on experimentally induced obesity in the rodent. *Am J Clin Nutr.* 1977 May;30(5):767-76.

Sullivan C, Triscari J. Novel pharmacological approaches to the treatment of obesity. In George A. Bray, ed., *Recent Advances in Obesity Research: II* (Westport, CT: Technomic Publishing Co., 1977) 442-452.

Sullivan AC, Dairman W, Triscari J. (–)-threo-Chlorocitric acid: a novel anorectic agent. *Pharmacol Biochem Behav.* 1981 Aug;15(2):303-10.

Sullivan, A.C., J. Triscari and L. Cheng. Appetite regulation by drugs and endogenous substances. In Myron Winick, ed., *Nutrition and Drugs* (New York: Wiley & Sons, 1983), 139-167. Also published as Sullivan AC, Triscari J, Cheng L. Appetite regulation by drugs and endogenous substances. *Curr Concepts Nutr.* 1983;12:139-67.

Sullivan, Ann C. and J. Triscari. Pharmacologic approaches to the regulation of metabolism and obesity. In Jules Hirsch and Theodore B. Van Itallie, eds., *Recent Advances in Obesity Research: IV* (London: John Libbey, 1983) 196-207.

Sullivan AC, Gruen RK. Mechanisms of appetite modulation by drugs. *Fed Proc.* 1985 Jan;44(1 Pt 1):139-44.

Triscari J, Sullivan AC. Studies on the mechanism of action of a novel anorectic agent, (–)-threo-chlorocitric acid. *Pharmacol Biochem Behav.* 1981 Aug;15(2):311-8.

It has now been demonstrated experimentally that the Roche position that HCA suppresses appetite through vagal afferents associated with the liver almost certainly is mistaken. In an animal trial in which the hepatic branch of the vagus was severed (hepatic branch vagotomy), there was no significant effect found with this surgery in comparison with controls. (Leonhardt M, Langhans W. Effect of hydroxycitrate on food intake and body weight regain in rats after hepatic branch vagotomy or sham vagotomy. Society for the Study of Ingestive Behavior, Annual Meeting 2001.)

Very recent papers have cast no more light on the anorectic effects of HCA. One research team that looked into the effects of HCA on serum leptin and insulin levels in mice had no new insights other than to suggest that HCA displays leptin-like activity, a point that the inventors made several years ago and for which we hold United States Patent 6,476,071. (Hayamizu K, et al. Effect of *Garcinia cambogia* extract on serum leptin and insulin in mice. *Fitoterapia*. 2003 Apr;74(3):267-73.) Another paper that directly confronts the issue says, “the anorectic mechanism of HCA is unknown.” (Leonhardt M, Langhans W. Hydroxycitrate has long-term effects on feeding behavior, body weight regain and metabolism after body weight loss in male rats. *J Nutr*. 2002 Jul;132(7):1977-82.)

Yet another recent study suggests that HCA acts by means of influencing serotonergic mechanisms. This conclusion appears to be based on in vitro data, to wit: “[HCA] can inhibit [3H]-5-HT uptake (and also increase 5-HT availability) in isolated rat brain cortical slices in a manner similar to that of SRRIs, and thus may prove beneficial in controlling appetite, as well as treatment of depression, insomnia, migraine headaches and other serotonin-deficient conditions.” (Ohia SE, et al. Safety and mechanism of appetite suppression by a novel hydroxycitric acid extract (HCA-SX). *Mol Cell Biochem*. 2002 Sep;238(1-2):89-103.) Note that these conclusions and speculations do not touch on gastric emptying. Moreover, they are based on inappropriate extrapolations. Some early preliminary work showed that labeled ¹⁴C attached to HCA found its way into the brain. (Sullivan C, Triscari J. Metabolic regulation as a control for lipid disorders. I. Influence of (–)-hydroxycitrate on experimentally induced obesity in the rodent. *Am J Clin Nutr*. 1977 May;30(5):767-76.) However, work published by the same authors at a later date indicated otherwise. “Hydroxycitrate, chlorocitrate, and epoxyaconitate, compounds that are structurally similar to the tricarboxylic acid cycle intermediate citric acid, but that differ markedly

in biochemical activity, have recently been evaluated in animals for effects on appetite. Because neither these compounds nor their metabolites enter the brain, their primary effects on food intake occur by peripheral mechanisms.” (Sullivan AC, Gruen RK. Mechanisms of appetite modulation by drugs. Fed Proc. 1985 Jan;44(1 Pt 1):139-44.) Again, it is well known that peripheral serotonin is metabolized virtually entirely peripherally. Indeed, this fact led to great concern when the compound 5-HTP (5-hydroxytryptophan extracted from the seeds of *Griffonia simplicifolia*) was first introduced as a dietary supplement. Moreover, even in the rat brain slices, it is likely that citrate would have yielded the same results as did HCA inasmuch as this was found to be the case in earlier brain slice experiments looking at acetylcholine production. (Tucek S, Dolezal V, Sullivan AC. Inhibition of the synthesis of acetylcholine in rat brain slices by (-)-hydroxycitrate and citrate. J Neurochem. 1981 Apr;36(4):1331-7.)

In any event, the same mistakes are made by the same authors in Ohia, Sunny E. et al., June 26, 2003, United States Patent Application 20030119913 (also available as WO 03/053454). Moreover, even had Ohia, et al. not relied on rat brain slices, but rather on direct blood tests in humans, their suggestion as the anorectic impact of serotonin from the ingestion of HCA would not have had significance with regard to gastric emptying. Several sets of researchers have demonstrated that serotonin, either locally or centrally, probably is not the major agent in the control of gastric emptying. (Chial HJ, et al. Selective effects of serotonergic psychoactive agents on gastrointestinal functions in health. Am J Physiol Gastrointest Liver Physiol. 2003 Jan;284(1):G130-7.) (Hansen L, Holst JJ. The effects of duodenal peptides on glucagon-like peptide-1 secretion from the ileum. A duodeno-ileal loop? Regul Pept. 2002 Dec 31;110(1):39-45.) While there is evidence of the expression of 5-HT receptors by extrinsic duodenal afferents, both vagal and spinal, that can be blocked by some (but not all) antagonists to reduce the inhibition of gastric emptying induced by glucose and mannitol, attempts to increase this gastric inhibitory effect via 5-HT agonists have not met with success. Indeed, increased levels of 5-HT in the gut tend to be associated not with delayed gastric emptying, but rather with irritable bowel syndrome (IBS) and diarrhea. The recommendation in such cases? 5-HT antagonists. (Chey WD. Tegaserod and other serotonergic agents: what is the evidence? Rev Gastroenterol Disord. 2003;3 Suppl 2:S35-40.)

Very recent reviews of the chemistry and biochemistry have added little insight to the

anorectic and weight loss actions of HCA. One such review, following recent research, argues that the inhibition of ATP:citrate lyase by HCA markedly diminishes the cellular pool of malonyl-CoA, indicating that citrate was the major substrate for the malonyl-CoA precursor, that is, cytosolic acetyl-CoA. There is sufficient evidence that because HCA inhibits ATP:citrate lyase, it also acts to limit the pool of cytosolic acetyl-CoA, the precursor of malonyl-CoA. This type of regulation of the malonyl-CoA level may affect the signaling of fuel status in hypothalamic neurons regulating feeding behavior. In the opinion of this review, these findings lend support to the theory that HCA may represent a biochemical target for the control of appetite/feeding behavior and body weight, by acting at the metabolic level and not directly via the central nervous system as do classical appetite depressants. (Jena BS, Jayaprakasha GK, Singh RP, Sakariah KK. Chemistry and biochemistry of (-)-hydroxycitric acid from *Garcinia*. J Agric Food Chem. 2002 Jan 2;50(1):10-22.) Noteworthy is the fact that this review does not even consider issues of gastric emptying or short-term actions by HCA on gastric motility.

That there are quite serious difficulties with the present use of HCA as a weight loss agent is obvious from readily available published data. US and European trials have cast doubt on its efficacy. In part, this may be due to the salts used in the trials. Of the readily available forms of HCA, only the potassium and sodium salts of HCA are absorbed well enough to be effective agents at tolerable levels of ingestion. In the experience of the present inventors, the calcium salts of HCA are markedly inferior to the potassium salt, and even including calcium as part of a potassium salt to form a double metal salt which is more workable than is the hygroscopic pure potassium salt at the same time significantly reduces efficacy. Several derivatives of HCA may also be active and effective. (United States Patents 3,993,668; 3,919,254; 3,767,678.) However, liquid forms of HCA currently in use are irritating to the digestive system, depending upon the dose, and may cause an elevation of stress hormones as a result. Researchers have found that animals given high doses of the liquid form of the compound orally exhibit stress behavior. (Ishihara K, Oyaizu S, Onuki K, Lim K, Fushiki T. Chronic (-)-hydroxycitrate administration spares carbohydrate utilization and promotes lipid oxidation during exercise in mice. J Nutr. 2000 Dec;130(12):2990-5.) Similarly, the ethylenediamine salts of HCA used in some of the later research performed by Hoffman-La Roche are known to be irritating and even toxic, properties which are due to the ethylenediamine ligand and not to the HCA.

All of the more recent and more thorough clinical trials on HCA not only have failed to produce appetite suppression, but also—and surprisingly in light of the claims made in several HCA patents already granted—have produced trends toward weight gain in some instances. (Heymsfield SB, Allison DB, Vasselli JR, Pietrobelli A, Greenfield D, Nunez C. *Garcinia cambogia* (hydroxycitric acid) as a potential antiobesity agent: a randomized controlled trial. *JAMA*. 1998;280:1596-1600; Mattes RD, Bormann L. Effects of (-)-hydroxycitric acid on appetitive variables. *Physiol Behav*. 2000 Oct 1;71(1-2):87-94.) Although they did not pursue the matter thoroughly, two Roche researchers in 1977 showed that HCA in the cytosol of the cell will activate acetyl CoA carboxylase similarly to the citrate it resembles. The effect of this property is that in diets which supply a source of acetyl CoA to the cytosol other than via citrate derived from the mitochondria, which means diets containing appreciable amounts of fat or alcohol as opposed to diets consisting almost exclusively of carbohydrates, HCA may increase the synthesis of fats and weight gain. (Triscari J, Sullivan AC. Comparative effects of (-)-hydroxycitrate and (+)-*allo*-hydroxycitrate on acetyl CoA carboxylase and fatty acid and cholesterol synthesis *in vivo*. *Lipids* April 1977;12(4): 357-363.) Not a single one of the patents which have been granted to date for the employment of HCA as an antiobesity agent (United States Patents 3,764,692; 5,626,849; 5,783,603; 5,914,326 and others proposing the use of HCA as an adjunctive ingredient) has indicated any awareness of its paradoxical effects, effects that have led to either null or negative results in the major clinical trials with HCA up to the point of this writing.

The inventors have found that HCA exerts several quite distinct effects and that “reverse effects” can be triggered by dose amounts and/or dosing patterns that are inappropriate to match diet and other factors. The discovery described here is that HCA delays gastric emptying. We have described elsewhere findings that the weight loss attributable to lessened food intake can be distinguished analytically from weight loss which appears related to changes in metabolism and that the anorectic effects of HCA do not normally last beyond approximately 7 weeks. We have further noted that higher fat (and alcohol) diets require higher dosages of HCA. Moreover, inadequate dosages of HCA can lead to weight gain. (See US Patent 6,476,071 and also US Patent Application No. 10/616,321 entitled “Treating Cachexia and Excessive Catabolism with (-)-Hydroxycitric Acid.”)

Roche maintained that the minimum effective doses of HCA in rats on a low fat diet (using trisodiumhydroxycitrate as the salt) are 2.63 mmoles/kg once per day or 0.33 mmoles/kg twice per day. When added to food, the typical dosage used by Roche was 52.6 mmol /kg feed. All subsequent individuals and groups working with the compound accepted that it must be given either as one extremely massive dose or, preferably, as two or three smaller doses delivered 30 to 60 minutes prior to meals. The appetite control mechanism of HCA was said to stem from the increased production of glycogen and/or stimulation of glucoreceptors in the liver, either of which results in satiety through signals sent to the brain via the hepatic branch of the vagus nerve. Roche researchers over a period of many years repeatedly maintained that HCA does not influence gastric emptying. No one ever challenged Roche on these matters despite the now more than a decade of free sale of HCA products within the United States and abroad.

The inventors, however, realized quite early that the Roche procedure and explanations do not fit the observable data regarding HCA. Our evidence and reasoning are as follows:

1) Roche claimed that glucoreceptors in the liver become more active because of HCA and that there must be a further step of signalling the brain. However, this suggests that there should be a considerable time lag before appetite suppression appears inasmuch as food must exit the stomach and glucose must reach the liver before an effect appears. Our experience, to the contrary, is that under certain conditions the anorectic effect of HCA appears extremely rapidly. This is in line with the actions of glucagon-like peptide (GLP-1) and/or other regulators of gastric emptying, but not typical of serotogenic regulation.

2) Roche's position on HCA implies that the glucoreceptors must be "primed" by a previous meal in order for HCA to work well — no glycogen, no anorexia. To the contrary, we found that such priming is not necessary. Although no priming is necessary, a "preload" is. This means that there must be food or volume in the stomach for HCA to work, as one would expect with an inhibitor of gastric emptying.

3) Whereas Roche focused on the putative role of the liver in the satiety associated with HCA, the inventors are more impressed by the fact that *de novo lipogenesis* also occurs in tissues of the

small intestine. This suggests that just as there are early sensing glucoreceptors in the duodenal mucosa which activate glucagon like peptide-1 (GLP-1) upon saturation with glucose and certain other sugars, one might expect that the presence of HCA to lead to the release of GLP-1 inasmuch as HCA to the cellular machinery looks like the citrate that is generated from excess glucose.

4) Roche performed experiments in which the ventromedial hypothalamus (VMH, the so-called satiety center) was destroyed, yet HCA nevertheless maintained its appetite suppression. (Sullivan C, Triscari J. Metabolic regulation as a control for lipid disorders. I. Influence of (-)-hydroxycitrate on experimentally induced obesity in the rodent. *Am J Clin Nutr.* 1977 May;30(5):767-76.) The widely accepted theory is that the obese animal eats more because it releases less of the satiety-inducing neurotransmitter serotonin in the hypothalamus. This Roche experiment indicates that HCA a) does not require an intact VMH and b) probably does not require the actions of serotonin in the brain.

5) Initially, Roche tied weight loss and decreased food consumption together, and it later only partially retreated from this stance. However, the company's own data showed that at the end of 80 days, there was a 4% net reduction in food intake compared with controls, yet a 78% reduction in weight gain. (Sullivan C, Triscari J. Metabolic regulation as a control for lipid disorders. I. Influence of (-)-hydroxycitrate on experimentally induced obesity in the rodent. *Am J Clin Nutr.* 1977 May;30(5):767-76.) Moreover, in a pair-feeding study, the HCA-fed rats gained substantially less weight than did controls limited to the same food intake. (Greenwood MR, Cleary MP, Gruen R, Blase D, Stern JS, Triscari J, Sullivan AC. Effect of (-)-hydroxycitrate on development of obesity in the Zucker obese rat. *Am J Physiol.* 1981 Jan;240(1):E72-8.) The inventors' own animal trials demonstrated that the reduction in food intake was not tightly linked to a reduction in weight gain.

6) Human trials have yielded results that indicate clearly that the appetite suppression found with HCA is only weakly related to weight loss. On the one hand, in a trial published in 2002, although food intake decreased 15-30%, there was no significant weight loss over 2 weeks.

(Westerterp-Plantenga MS, Kovacs EM. The effect of (-)-hydroxycitrate on energy intake and satiety in overweight humans. *Int J Obes Relat Metab Disord*. 2002 Jun;26(6):870-2.) In this case, the ingestion of 300 mg three times daily HCA from SuperCitriMax potassium (16%) calcium (11%) hydroxycitrate led to only a trend toward weight loss despite the very large decrease in caloric intake. The inventors have noted elsewhere, for instance our US Patent 6,476,071, that ingesting too little HCA can even cause weight gain, probably due to the activation of acetyl-CoA carboxylase. The inventors also regard the delivery via tomato juice in this study is very important. This juice is acid, hence even the calcium salt of HCA dissolves fully in it, yet the juice does not contain components that rapidly bind to the HCA. Moreover, supplies adequate sugars to activate gut responses and the juice is extremely rich in potassium — much more so than, say, orange juice. As we have indicated in other patents, the potassium/calcium salt of HCA is not well absorbed in the small intestine and therefore the metabolic effect of SuperCitrimax, as is true of all similar calcium and potassium-calcium salts of HCA, is weak in comparison with effect of a fully reacted potassium salt. The 2-week period of this study was inadequate for the metabolic intervention to manifest.

On the other hand, 1,200 mg HCA daily given as tablets (2 x 400 mg 50% material as Citrin® calcium hydroxycitrate taken 3 times daily before meals) given for 12 weeks led to significant weight loss despite no significant change in food intake. The findings were 3.7 ± 3.1 kg active versus 2.4 ± 2.9 kg placebo. Over a 3 month period, these results of less than a pound of additional weight loss per month are hardly impressive; however, the difference is significant. (Mattes RD, Bormann L. Effects of (-)-hydroxycitric acid on appetitive variables. *Physiol Behav*. 2000 Oct 1-15;71(1-2):87-94.) The inventors have demonstrated elsewhere that calcium hydroxycitrate is not well absorbed, yet the longer time frame in this study allowed for a metabolic effect despite no significant anorectic effect. Animal trials using very high dosages of HCA have shown an elevation in energy expenditure.

These clinical trials are evidence that the anorectic effects of HCA should be considered as being separable from its weight loss effects in humans just as animal trials indicate that this is the case in other species.

7) The inventors were the first researchers to explore the use of controlled delivery techniques

with HCA to bypass release into the stomach and have received patent protection in this regard. We employed controlled delivery in a pertinent example found in our US Patent 6,207,714 covering the use of HCA for blood glucose and insulin metabolism. At that time and as a result of our experiments, we discovered that the release into the small intestine, although it could have a profound effect on blood sugar, had only a small impact on appetite. This confirmed our intuition that increasing blood levels of HCA via enteric delivery so as to potentiate the many metabolic benefits of the compound could be at least partially divorced from the appetite suppressing actions of the substance.

8) The inventors have explored the interaction of HCA with a number of other compounds. In a pilot study, we discovered that the consumption of hot peppers, for instance, can nullify the immediate anorectic actions of HCA. These results are in line with published studies demonstrating that capsaicin increases the rate of gastric emptying. (Debrececi A, Abdel-Salam OM, Figler M, Juricskay I, Szolcsanyi J, Mozsik G. Capsaicin increases gastric emptying rate in healthy human subjects measured by ¹³C-labeled octanoic acid breath test. *J Physiol Paris*. 1999 Nov;93(5):455-60.)

9) As noted above, HCA is protective against the ulcerative actions of alcohol and indomethacin. Experimentally, it has been shown that capsaicin-sensitive sensory nerves are involved in ulcerations from these sources and that pre-treatment with capsaicin attenuates the gastric protection afforded by, for example, the oleanolic acid oligoglycoside momordin Ic. (Matsuda H, Li Y, Yoshikawa M. Roles of capsaicin-sensitive sensory nerves, endogenous nitric oxide, sulfhydryls, and prostaglandins in gastroprotection by momordin Ic, an oleanolic acid oligoglycoside, on ethanol-induced gastric mucosal lesions in rats. *Life Sci*. 1999;65(2):PL27-32.) A link is thus established between the gastro-protective properties of HCA and the gastric motility inhibiting property of the compound. Quite obviously, the dosage prescriptions of Roche and the use of HCA in weight loss have no bearing here.

The explanations for the satiation induced by HCA championed by Roche and until now universally embraced cannot any longer be accepted. Direct experimentation in rats has shown

that hepatic vagal afferents probably are not involved, albeit gastric branch vagal afferents may be implicated. (Kaplan JM, Siemers WH, Smedh U, Schwartz GJ, Grill HJ. Gastric branch vagotomy and gastric emptying during and after intragastric infusion of glucose. *Am J Physiol*. 1997 Nov;273(5 Pt 2):R1786-92.) Clinical trials have shown the HCA can induce a quite massive reduction in food intake with only a minor trend in change in body weight or, vice versa, no significant reduction food intake, yet a significant loss of weight. The inventor's own tests have shown that the release point for HCA, i.e., whether stomach or intestine, is a determining factor in these results. In our experience, the appropriate delivery method will induce the feeling of fullness in the stomach at one sitting without any requirement of carbohydrate preloading and without resort to massive doses of HCA. One implication of this knowledge is that HCA can be used for the treatment of conditions related to gastric emptying, but unrelated to weight loss.

In our US Patent 6,476,071 we showed that HCA lowers leptin levels. This result subsequently has been confirmed by others and has led on group of researchers to refer to a "leptin-like" effect with HCA. This may be of relevance in light of contemporary research into gastric emptying. Cholecystokinin (CCK) is a major gastrointestinal neuropeptide that is secreted in response to food ingestion. It is involved in the feedback regulation of gastric emptying and also modulates food intake. Leptin, a hormone that regulates food intake and energy balance, is secreted from adipose tissue, gastric mucosa, fundic glands, and other tissues. The gastric effects of leptin activate the brain stem nucleus tractus solitarius (NTS) neurons that respond to gastric vagal stimulation. The distal stomach containing the pylorus determined CCK gastric activity, whereas both the proximal and distal stomach are important for leptin's effect. (Yuan CS, Attele AS, Dey L, Xie JT. Gastric effects of cholecystokinin and its interaction with leptin on brainstem neuronal activity in neonatal rats. *J Pharmacol Exp Ther*. 2000 Oct;295(1):177-82.) In light of the inventors' own experiments involving HCA and the loss of its satiety effect with the ingestion of hot peppers, it is supportive to find in the literature work on the existence of a functional synergistic interaction between leptin and CCK leading to early suppression of food intake involving CCK-A receptors and capsaicin-sensitive afferent fibers. (Barrachina MD, Martinez V, Wang L, Wei JY, Tache Y. Synergistic interaction between leptin and cholecystokinin to reduce short-term food intake in lean mice. *Proc Natl Acad Sci U S A*. 1997 Sep 16;94(19):10455-60.) As can be seen, research indicates that receptors controlling

gastric emptying can be found in the stomach itself. It is probable that HCA acts on one or more sets of these receptors to influence CCK release or receptor activation.

Many gut-produced and released compounds act upon the brain both via vagal afferents and directly. Gastric distention by itself may activate these systems, again, both locally and in the brain. For instance, above it was noted that gastric leptin activates the brain stem nucleus tractus solitarius (NTS) neurons that respond to gastric vagal stimulation. Similarly, a group of neurons in the caudal nucleus of the solitary tract processes preproglucagon to glucagon-like peptides (GLP)-1 and -2, peptides that inhibit food intake when administered intracerebroventricularly. Significantly, gastric distension that may be considered within the physiological range activates GLP-1/2-containing neurons, suggesting some role in normal satiety. (Vrang N, Phifer CB, Corkern MM, Berthoud HR. Gastric distension induces c-Fos in medullary GLP-1/2-containing neurons. *Am J Physiol Regul Integr Comp Physiol*. 2003 Aug;285(2):R470-8. Epub 2003 Apr 24.) In turn, despite its effect on gastric emptying, GLP-1 does not lead to postprandial discomfort because, in part, it allows for gastric accommodation. (Delgado-Aros S, Vella A, Camilleri M, Low PA, Burton DD, Thomforde GM, Stephens D. Effects of glucagon-like peptide-1 and feeding on gastric volumes in diabetes mellitus with cardio-vagal dysfunction. *Neurogastroenterol Motil*. 2003 Aug;15(4):435-43.) Despite its many insulin-related effects found at elevated dosages, research findings suggest a primarily inhibitory function for GLP-1 involving ileal brake mechanisms. (Nauck MA, Niedereichholz U, Ettler R, Holst JJ, Orskov C, Ritzel R, Schmiegeler WH. Glucagon-like peptide 1 inhibition of gastric emptying outweighs its insulinotropic effects in healthy humans. *Am J Physiol*. 1997 Nov;273(5 Pt 1):E981-8.) Because HCA delays gastric emptying and increases gastric volume, there is little question but that HCA also causes the release of GLP-1. The real issue is one of chicken-or-egg. Something similar might be said of the other incretin, gastric inhibitory polypeptide (GIP).

An indication that HCA likely does increase GLP-1 comes from a study with the organic acid sodium propionate which delayed gastric emptying with a pasta meal and increased the levels of GLP-1. (Frost GS, Brynes AE, Dhillon WS, Bloom SR, McBurney MI. The effects of fiber enrichment of pasta and fat content on gastric emptying, GLP-1, glucose, and insulin responses to a meal. *Eur J Clin Nutr*. 2003 Feb;57(2):293-8.) Furthermore, whereas Roche focused on the putative role of the liver in the satiety associated with HCA, the inventors emphasize the fact

that *de novo lipogenesis* also occurs in tissues of the small intestine. This fact is generally overlooked and suggests, as was pointed out above, that just as there are early sensing glucoreceptors in the duodenal mucosa which activate GLP-1 upon saturation with glucose and certain other sugars, one might expect that the presence of HCA to lead to the release of GLP-1 inasmuch as HCA to the cellular machinery looks like the citrate that is generated from excess glucose.

In the end, it is also promising to return to CCK. Studies in humans have repeatedly shown that CCK inhibits food intake. However, a gastric preload is generally necessary to achieve a satiating effect with CCK. Thus, CCK given at physiologically relevant concentrations to fasting humans had no effect on satiety or food intake, while the same infusion rate after a banana preload decreased food intake. (Hellstrom PM, Naslund E. Interactions between gastric emptying and satiety, with special reference to glucagon-like peptide-1. *Physiol Behav.* 2001 Nov-Dec;74(4-5):735-41.) This pattern appears to describe the actions of HCA quite well. The compound does not inhibit food intake by itself on an empty stomach, but rather requires food to work. Hence, the inventors argue that HCA acts in part upon CCK receptors in line with recent research findings that the requirement for a negative charge at the CCK-A receptor provided in the natural substrate by a sulfate group can be satisfied by organic acids. (Tilley JW, Danho W, Lovey K, Wagner R, Swistok J, Makofske R, Michalewsky J, Triscari J, Nelson D, Weatherford S. Carboxylic acids and tetrazoles as isosteric replacements for sulfate in cholecystokinin analogues. *J Med Chem.* 1991 Mar;34(3):1125-36.) CCK acts upon receptors in the stomach, but it is known, as well, to act upon duodenal mucosal receptors which, as noted earlier with HCA, feed to afferents that are sensitive to capsaicin. Research supports the notion that acid inhibitors of gastric emptying are not influenced by serotonin blockade and are enhanced by the presence of sugars.

It is possible to enhance the gastric inhibitory effects of HCA through a variety of means, especially if the compound can be given as part of a foodstuff. Citric acid, sodium citrate and other related compounds should further contribute to inhibiting gastric emptying. (Shiotani A, Saeed A, Yamaoka Y, Osato MS, Klein PD, Graham DY. Citric acid-enhanced *Helicobacter pylori* urease activity in vivo is unrelated to gastric emptying. *Aliment Pharmacol Ther.* 2001 Nov;15(11):1763-7.) In general, lowering pH has a systematic effect in delaying the onset of

gastric emptying and increasing gastric residence time. (Chaw CS, Yazaki E, Evans DF. The effect of pH change on the gastric emptying of liquids measured by electrical impedance tomography and pH-sensitive radiotelemetry capsule. Int J Pharm. 2001 Oct 4;227(1-2):167-75.) Similar actions can be expected from sodium propionate, propionic acid, gallic acid and propyl gallate. As discussed above in regard to one study employing HCA, delaying gastric emptying with these organic acids does not necessarily lead to weight loss. In comparative trials using HCA and citrate, the citrate did not have a significant impact upon weight.

A number of plant compounds and extracts have shown the ability to inhibit gastric emptying. These include extracts of marigold (*Calendula officinalis*), escins and other compounds from *Aesculus hippocastanum* seeds, extracts of the fruit of *Kochia scoparia*, and the roots and other parts of *Aralia elata*, proteinase inhibitor extracts from potato and soybean sources, and a variety of oleanolic acid glycosides from many sources. Other putative delayers of gastric emptying include herbal combinations such as one consisting of yerba mate, damiana and guarana.

No prior art suggests that HCA can be used to delay gastric emptying, that it influences glucagon-like peptides (GLP-1/2) nor that it influences cholecystokinin (CCK). Indeed, just the opposite is the case. The primary researchers repeatedly argued — wrongly — over an extended period of years in numerous journal articles and books that only an indirect mechanism based upon the liver is involved. All other parties involved in the research and sale of HCA have similarly overlooked its impact on gastric emptying. The present inventors are the first to recognize not only that HCA delays gastric emptying, but also that this allows for the introduction of quite new dosage schedules and the use of HCA in novel areas unrelated to weight loss, such as forms of hypertension, liver dysfunction, and so forth and so on.

SUMMARY OF THE INVENTION

The inventors have discovered that food and pharmaceutical compositions containing (–)-hydroxycitric acid, its salts, amides and esters can be employed for delaying gastric emptying and increasing receptive relaxation for preventing and treating diverse conditions. There are concomitant influences on glucagon-like peptides (GLP-1/2) and cholecystokinin (CCK). Altered gastric emptying and accommodation are found with forms of hypertension, liver dysfunction

and gastrointestinal ulcers, especially duodenal ulcer. Numerous medications, such as antibiotics (erythromycin, indomethacin, etc.) and including even some diet drugs (e.g., Orlistat and other lipase inhibitors), can accelerate gastric emptying. Surgery, such as for peptic ulcers, itself can lead to clinical dumping syndrome, as can other types of surgery performed on the stomach. Other factors or conditions that lead to acceleration of gastric emptying include obesity, high-energy density of food, fat intolerance, early stages of noninsulin-dependent diabetes mellitus, Zollinger-Ellison syndrome, and intermittent episodes in other forms of diabetes. HCA delivered in the form of its potassium salt is efficacious at singly delivered dosages of between 150 mg and 5 grams, preferably at a dosage of between 500 mg and 3 grams for most individuals. Other salts, amides and esters are active at individual dosage ranges, with, for instance, the sodium salt acting similarly to the potassium salt whereas salts containing calcium are less active. Various delivery methods that preferentially expose HCA to stomach and duodenal receptors and sensors without undue binding of the compound to inactivating substances are provided.

Objects and Advantages

It is an objective of the present invention to provide methods for employing food and pharmaceutical compositions containing (–)-hydroxycitric acid, its salts, amides and esters to delay gastric emptying and increase receptive relaxation. Further novel objects and advantages include the employment of HCA in conditions such as presinusoidal portal hypertension, liver cirrhosis, duodenal ulcer, dumping syndrome, accelerated gastric emptying due to drugs (antibiotics, lipase inhibitors, etc.), rapid gastric emptying due to pre-diabetic and diabetic conditions, and various other circumstances described above. These objects and advantages are not derived from the anorectic actions commonly claimed for the use of HCA as an anti-obesity agent, but rather depend upon mechanisms not heretofore elucidated.

Moreover, these objects and advantages do not require adherence to current dosage regimens. Current recommendations for the use of HCA require that it be ingested either two or three times per day 30 to 60 minutes prior to meals for weight loss. However, such a regimen may be of little benefit in conditions such as those involving duodenal ulcers or gastric lesions where extended residence time for HCA in contact with the stomach is desirable. Similarly, current recommendations for the use of HCA may not benefit those suffering from drug- or surgery-

induced dumping or rapid gastric emptying.

Yet a further advantage of the present discovery is that it allows for dramatic improvements in the use of HCA in the field of bariatrics. HCA can now be used to overcome at least some of the side effects of weight loss drugs such as Orlistat. Through the use of the present invention it is also possible to overcome the primary impediment to the successful employment of HCA for weight loss during the first two months of use and achieve consistent results in humans, something not evidenced in published clinical trials performed in the United States and Europe.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The free acid form and various salts of (–)-hydroxycitric acid (calcium, magnesium, potassium, sodium and mixtures of these) have been available commercially for several years. Any of these materials can be used to fulfill the invention revealed here, but with varying degrees of success. These materials are generally useful in this descending order of efficacy: potassium salt, sodium salt, free acid, magnesium salt, calcium salt. The previously patented hydroxycitric acid derivatives (mostly amides and esters of hydroxycitric acid, the patents for which are now expired) likely are roughly equivalent to the HCA sodium salt in efficacy.

The discovery that the stomach and the duodenum are the primary sites of action of HCA in delaying gastric emptying is of great importance. Also significant is the fact that the delivery of HCA after the meal, which is to say after the stomach has already begun to empty, is non-productive in this regard. Yet another factor that needs to be taken into the account is the cooperation between HCA and sugars (digestible and many non-digestible, e.g., xylitol) and similar compounds in gastric signalling. Finally, it must be kept clearly in mind that capsaicin and substances similar to capsaicin in their effects upon gastric vagal afferents and other capsaicin-sensitive afferents will nullify the potential of HCA for delaying gastric emptying.

Desirable deliveries must take into account that HCA binds to many gums, fibers, anthocyanins, catechins and other compounds. Color changes in tea and grape juice when salts of HCA are added are immediately visible signals indicating that unwanted changes that are taking place. Insoluble salts, such as calcium (–)-hydroxycitrate, when delivered as tablets or even as capsules may not fully dissolve early enough in the stomach to be efficacious. The inventor's consistent experience has been that calcium makes HCA less active even when present merely as

a component fraction of a potassium salt and used to make the potassium salt less hygroscopic (one of the so-called double metal salts). It may be that calcium blocks a potassium-dependent transport channel or otherwise interferes with the impact of HCA upon gastric emptying or even interferes with the metabolic effects of HCA when included as part of the salt. The free acid, similarly, is hard to work with because it lactonizes readily and the lactone is much less active than is the acid.

Methods for taking advantage of the present invention include, but are not limited to the following in addition to one or more sources of HCA. These items are intended to provide for “instant release” into the stomach, be released by chewing or upon exposure to stomach acid, and so forth. Employment of the salts of HCA that are most active in producing satiety (potassium and sodium) requires the concomitant application of one or more of the delivery methods (patented and patent-pending) developed by the inventors to render these hygroscopic salts workable. Examples given below elaborate and extend methods for

- 1) capsules or tablets containing sodium bicarbonate, potassium bicarbonate or (less advantageously) calcium carbonate sufficient to cause the rapid release of the contents of the capsule or tablet when exposed to stomach contents
- 2) capsules or tablets containing sodium bicarbonate or potassium bicarbonate plus alginic acid; also capsules or tablets containing sodium or potassium alginate to achieve a prolonged dwell time in the stomach and extended presentation to the stomach wall
- 3) dry packaged powders designed to mixed with water or juice and consumed between meals or prior to meals; HCA mixed into tomato juice is especially successful, whereas HCA tends to bind to components in citrus juices; precoating of the HCA with hydrophobic components is necessary before placing the salts in packaged materials
- 4) special processing of HCA salts, etc., for instance, with molten oils such as hydrogenated vegetable oil, glycerol monostearate, cetyl alcohol, stearyl alcohol and various high viscosity grades of conjugated polyethylene glycol, d- α -tocopheryl polyethylene glycol succinate (TPGS) and similar compounds (see inventors’ US Patent Application 10/447,992), after which this material, now rendered non-hygroscopic and non-reactive, is further encased in gelatin, tapioca, gums/pectins, inulin, cellulose derivatives, etc., for inclusion in thick drinks,

soft-center bars and candies, pudding snacks, jelly-like confections, “gummy” deliveries, liquid meal replacements, etc.

- 5) the inclusion with or use in conjunction with HCA of other agents that influence gastric emptying, such as citric acid, sodium or potassium citrate, other citric acid salts, sodium propionate, propionic acid, gallic acid, propyl gallate; extracts of marigold (*Calendula officinalis*); escins and other compounds from *Aesculus hippocastanum* seeds; extracts of the fruit of *Kochia scoparia*, and the roots and other parts of *Aralia elata*; saponins, especially theasaponin E1 from the the seeds of the tea plant (*Camellia sinensis* L.); extracts from bay leaf (*Laurus nobilis*), especially costunolide and its active component, alpha-methylene-gamma-butyrolactone (*alpha*-MGBL); proteinase inhibitor extracts from potato and soybean sources; a variety of oleanolic acid glycosides from many sources; also herbal combinations such as one consisting of yerba mate, damiana and guarana.

The following are examples of supporting data and means of application for the invention.

EXAMPLE 1

UNEXPECTED REPORTS OF DELAYED GASTRIC EMPTYING

Evidence that HCA during the initial weeks of usage likely reduces appetite through an effect upon gastric emptying emerged from a clinical trial. Previously, Roche, in public documents, had shown that HCA can control food intake if administered in one large bolus dose or in two much smaller dosages given prior to meals. This can be interpreted either as indicating the clearance rate of the drug or as indicating a mechanism. HCA given after a meal has already begun has no impact upon food intake; the stomach must become again completely empty before anorexia returns. However, HCA given continuously in the food supply to rats, animals which eat more or less continuously during waking hours if food is available will, again, reduce food intake. Roche argued in numerous public documents that the appetite suppressing actions of HCA depend upon the activation of glucoreceptors in the liver, yet this particular explanation for a peripherally-acting agent (no effect upon the central nervous system) seems inadequate in light of the very quick onset of satiety after a meal has begun in experiments in which animals are restricted to two meals per day after gavage with the compound. It also seems to be inadequate given that in an experiment in which the rats' satiety center of the brain had been destroyed there

still was appetite suppression. Hepatic glucoreceptor activation of the vagus nerve probably would have no impact upon the satiety center of the brain under such circumstances. Hence, meals clearly trigger some mechanism which has been activated by HCA. Moreover, it is unlikely that sufficient calories from a meal can reach the liver in time to account for the rapid onset of satiety or satisfy these other conditions just mentioned. In a drug which acts at least in part upon receptors in the stomach and/or small intestine, these factors, however, would no longer be problematic.

Data on human usage emerged from a multi-week pilot open clinical weight loss trial with extremely obese patients which was undertaken to gauge the effects of a pouch delivery form of a potassium salt of (–)-hydroxycitrate under the normal circumstances faced in clinical practice with this patient population. Sixteen patients were enrolled, three of whom were diabetics on medications and several others who were suspected of suffering from insulin resistance. The patients ingested 3 - 4 grams of HCA (in the form of the potassium salt) per day in two divided doses. Aside from being informed that they must eat a carbohydrate-containing meal within one hour of taking the HCA and that they should avoid eating late in the day, they were not instructed to follow any special diet or exercise plan outside their normal habits and no caloric restriction was imposed. This particular form of potassium (–)-hydroxycitrate delivery typically was mixed into water or juice and consumed at mid-morning and mid-afternoon. The delivery was a water-soluble immediate release form. It was a pre-commercial preparation and nearly all of the patients complained regarding the inconvenience and poor taste of the product, albeit there were no other issues of tolerability.

A number of patients continued on the program for 6 weeks. However, most patient data was good for only 3 weeks because two of the diagnosed diabetics experienced severe hypoglycemic reactions. Several other patients experienced good appetite suppression, yet also complained of episodic tiredness at the beginning of the program, a sign of low blood sugar. Two patients subsequently were placed on phentermine. One patient who followed the program for 10 weeks with excellent weight loss (32 pounds over 10 weeks) found that his tendency toward elevated blood sugar was stabilized during the program. This patient returned to his prior experiences of infrequent hypoglycemia roughly one week after he had left the program, something which suggests a carryover effect from the compound. The average weight loss over

the 3 week period for these 14 patients was approximately 3.1 pounds per person per week. In the eight patients with hypertension, the compound showed a surprising ability to normalize blood pressure. The clinical decision was made that potassium (–)-hydroxycitrate in an immediate release format can exercise a strong hypoglycemic effect in diabetics and that it appears to influence blood sugar levels in protodiabetics, as well. At therapeutically effective dosages, HCA probably should be used with diabetic populations only under a physician's care.

When questioned regarding degree of appetite suppression and compliance patterns in taking the HCA, many patients noted that not only did the compound make them “feel fuller faster,” but also that they seemed to feel full for a longer period of time. The authors speculated that rapidity of onset of satiety may involve intestinal glucoreceptors and that continued satiety could involve these same receptors or some allied mechanism. For instance, protease inhibitors which block trypsin and chymotrypsin may enhance satiety by preventing digestion of the cholecystokinin-releasing peptide (CCK-RP), a peptide which is secreted into the gut lumen during meals. CCK-RP can then stimulate release of the satiety peptide CCK from endocrine cells in the small intestine.

EXAMPLE 2

METABOLIC EFFECT WITHOUT APPETITE CONTROL

In Example 1, the HCA was delivered in an immediate-release preparation. Our unexpected findings with regard to blood sugar led us to question whether a relatively large dose of HCA might affect blood sugar levels in an individual whose blood sugar is in the low normal range. The inventors also were at the time experimenting with controlled delivery dosage forms and we sought to gather provisional data on such deliveries. A dose of 1.5 grams HCA derived from potassium (–)-hydroxycitrate and delivered in a special coated form designed to bypass interaction with stomach acids and to release only in the higher pH of the small intestine was used. After an overnight fast, the subject had a measured blood glucose level of 85 mg/dL. Subject ate a 500 calorie breakfast which included the experimental HCA. Two hours after this meal, subject's blood glucose level had dropped to 77 mg/dL. Subject reported no changes in energy levels, but this subject was known to metabolize fats well as fuel, hence was not expected to experience low energy. Striking at the time was the fact that delivery of potassium (–)-

hydroxycitrate to the small intestine and by-passing the stomach appeared to blunt the anorectic actions of the drug. This finding seemed paradoxical in that the outstanding metabolic effect, which might be thought to indicate blood levels of the drug, was not matched by even a normal level of feelings of fullness. This implied that at least part of the satiation induced by HCA comes about prior to entry of the compound into the blood. As noted in the text, studies published subsequent to our own research have shown the same pattern of at least partial disconnect between metabolic and appetite effects of HCA.

EXAMPLE 3

LEPTIN, A KNOWN LINK TO CHOLECYSTOKININ (CCK)

Very recently, Japanese researchers gave HCA to mice on a 10% sucrose diet and observed that levels of serum insulin and leptin as well as the leptin/white adipose tissue ratio were lower in the treated mice than in the control. They concluded that “these findings suggested that *G. cambogia* extract efficiently improved glucose metabolism and displayed leptin-like activity.” (Hayamizu K, Hirakawa H, Oikawa D, Nakanishi T, Takagi T, Tachibana T, Furuse M. Effect of *Garcinia cambogia* extract on serum leptin and insulin in mice. *Fitoterapia*. 2003 Apr;74(3):267-73.) The gastric effects of leptin activate the brain stem nucleus tractus solitarius (NTS) neurons that respond to gastric vagal stimulation. The distal stomach containing the pylorus determines CCK gastric activity, whereas both the proximal and distal stomach are important for leptin's effect. (Yuan CS, Attele AS, Dey L, Xie JT. Gastric effects of cholecystokinin and its interaction with leptin on brainstem neuronal activity in neonatal rats. *J Pharmacol Exp Ther*. 2000 Oct;295(1):177-82.) Various researchers have demonstrated an interaction between leptin and cholecystokinin. (Barrachina MD, Martinez V, Wang L, Wei JY, Tache Y. Synergistic interaction between leptin and cholecystokinin to reduce short-term food intake in lean mice. *Proc Natl Acad Sci U S A*. 1997 Sep 16;94(19):10455-60.)

In our US Patent 6,476,071 we showed that HCA alters insulin and leptin levels. When considered in the light of other evidence regarding the appetite suppression found with HCA, such findings provide reasonable evidence that HCA likely activates or interacts synergistically with CCK. In one study mentioned in our earlier patent, the inventors arranged for male OM rats aged 10 weeks to be fed a diet in which 30% of the calories were obtained from fat under

standard conditions. The rats were intubated twice daily with one of three HCA salts or placebo. The amount of HCA in each arm of 5 animals was the minimum dosage which had been found effective in the form of the pure trisodium salt of HCA in tests by Hoffmann-La Roche in animals ingesting a 70% glucose diet, i.e., 0.33 mmoles/kg body weight HCA given twice per day. The HCA salts used were these: CaKHCA = a mixed calcium and potassium HCA salt commercially marketed as being entirely water soluble; KHCA 1 = a relatively clean, but still hardly pure potassium salt of HCA with a good mineral ligand attachment supplying 4467 mg potassium / 100 grams of material; KHCA 2 = an impure potassium salt of HCA with large amounts of gums attached and poor mineral ligand attachment supplying 2169 mg potassium / 100 grams of material. Data was collected from the rat study with regard to serum insulin, leptin and corticosterone levels.

Group	Insulin ng/mL	Leptin ng/mL	Corticosterone ng/mL
Control	2.655	9.52	269.38
Control	7.077	18.94	497.87
Control	4.280	34.34	265.71
Control	9.425	24.32	209.54
Control	3.798	8.40	116.12
KHCA 1	3.880	9.93	45.79
KHCA 1	4.399	7.31	33.10
KHCA 1	3.181	9.25	65.57
KHCA 1	3.210	24.36	55.40
KHCA 1	3.639	9.07	84.62
KHCA 2	4.427	9.13	26.02
KHCA 2	4.301	9.75	270.83
KHCA 2	3.245	8.00	45.44
KHCA 2	3.695	9.16	45.63
KHCA 2	2.053	8.26	38.04

Both of the potassium (–)-hydroxycitrate arms were superior to the calcium/potassium arm (data not shown here) in reducing insulin, leptin and corticosterone concentrations. Because of the difficulty in achieving significance with only 5 data points per arm, calculations regarding insulin and leptin combined the data from the two KHCA arms. With respect to insulin, the one-tailed *P* value was a significant 0.0306, and the two-tailed *P* value fell slightly short of significance at 0.0612. Using this combined data, there was also a significant one-tailed *P* value difference between the two KHCA arms and the result found with the CaKHCA. With respect to leptin, the two KHCA arms were combined, in part, because of one anomalously high data point and yielded a one-tailed *P* value which was a significant 0.0241 and a two-tailed *P* value which was significant at 0.0482. Corticosterone results were highly significant even at 5 data points per arm. KHCA 1 was easily significantly superior to control: the one-tailed *P* value was a highly significant 0.0048, and the two-tailed *P* value was a highly significant 0.0096.

The implication of these data is that HCA, if supplied in appropriate amounts, may be useful in reducing insulin levels and insulin resistance, leptin levels and leptin resistance, and elevated glucocorticoid levels. Therefore, the inventors' data supports a conclusion that HCA displays “leptin-like” activity. Moreover, the effect of HCA on leptin levels was significantly stronger with KHCA than with the double-metal calcium and potassium salt. This disparity was paralleled by the greater appetite/food intake and weight gain found with the double-metal calcium and potassium salt which, on the high-fat diet employed in this study, led to food intake and weight gain greater than that found in control. Hence, we have indirect evidence from our own study of a link between the ingestion of HCA and the regulation of components known to interact with leptin, in this case CCK. It is not yet known why or how calcium interferes with the actions of HCA when used as a cation.

EXAMPLE 4

CAPSAICIN DEFEATS HCA-INDUCED SATIETY

The research literature supports a functional synergistic interaction between leptin and CCK leading to early suppression of food intake involving CCK-A receptors and capsaicin-sensitive

afferent fibers. (Barrachina MD, Martinez V, Wang L, Wei JY, Tache Y. Synergistic interaction between leptin and cholecystokinin to reduce short-term food intake in lean mice. *Proc Natl Acad Sci U S A*. 1997 Sep 16;94(19):10455-60.) This research indicates that receptors controlling gastric emptying can be found in the stomach itself. Other work demonstrates that capsaicin increases the rate of gastric emptying. (Debrececi A, Abdel-Salam OM, Figler M, Juricskay I, Szolcsanyi J, Mozsik G. Capsaicin increases gastric emptying rate in healthy human subjects measured by ¹³C-labeled octanoic acid breath test. *J Physiol Paris*. 1999 Nov;93(5):455-60.)

To test whether there is a capsaicin-HCA interaction as is suggested by our proposed effect upon CCK, the inventors invited 5 individuals to consume approximately 2 grams potassium (–)-hydroxycitrate in water about an hour before a meal. The meal itself began with a soup course. The participants reported that they felt full very soon after beginning to consume the likewise savory, but non-spicy main portion of the meal. At this point, bottles of red pepper sauce were supplied and the sauce was applied liberally. Shortly thereafter, the participants found that they could “eat through” the previous feeling of fullness. As is true of Example 3, this provides indirect evidence that HCA acts upon a CCK-related mechanism in inducing satiety.

EXAMPLE 5

IMMEDIACY OF HCA-INDUCED SATIETY

Contrary to the conclusions in the scientific literature based upon rats studies, the inventors surmised that HCA’s satiety is related to the volume of stomach contents rather than to the number of calories that have been presented to the liver. It is known that glucagon-like peptide has two points of action; the first occurs almost immediately as food begins to be ingested and influences gastric emptying, whereas the second occurs only much later and influences the tenacity of the satiety. Again, the first action of GLP-1 may in part be in response to gastric extension and may lead to both direct and vagally-mediated effects in the brain. A gastric preload also is generally necessary to achieve a satiating effect with CCK. Thus, CCK given at physiologically relevant concentrations to fasting humans had no effect on satiety or food intake, while the same infusion rate after a banana preload decreased food intake. (Hellstrom PM, Naslund E. Interactions between gastric emptying and satiety, with special reference to

glucagon-like peptide-1. *Physiol Behav.* 2001 Nov-Dec;74(4-5):735-41.) In other words, gastric volume and the act of loading the stomach seem to be important both for the first mechanism associated with GLP-1 and for the anorectic effect of CCK.

The inventors reasoned that if HCA quickly intervenes to delay gastric emptying and the mechanisms involved do not involve glucose receptors in the liver, then even consuming a drink characterized by high volume, but relatively few calories might lead to satiety. To test this theory and the palatability of an HCA salt when mixed with various flavors, we asked 5 individuals to consume approximately 2 grams potassium (–)-hydroxycitrate mixed in sweetened lemonade-like drinks prior to a meal. Consumption of the drinks took place over the course of approximately one half hour and involved 16 - 24 ounces of fluid, but only about 200 calories. As is well established, beverages do not normally have great satiating power. Nevertheless, all the participants found that they were satiated soon after the meal began. This example strongly suggests that gastric emptying and quick-acting satiety mechanisms are brought into play by HCA.

EXAMPLE 6

DIRECT EVIDENCE OF DELAYED GASTRIC EMPTYING

The intuition of the inventors has been upheld by experimental data. Hoffmann-La Roche in the form of the inventors Guthrie, et al. in a patent filing covering the use of chlorocitric acids (United States Patents 4,312,885 and 4,443,619) teach in their Example 25 that the trisodium salt of (–)-hydroxycitric acid under experimental conditions more than doubled the contents of the stomach of rats after a three hour meal in comparison with the stomach contents found in the controls (1973 + 154 versus 884 + 145 dpm X 103, which is 223% of control). Radioactively tagged materials also allowed evaluation of the amount of glucose that had entered the blood; in the case of HCA, this was only 79% of control. The results found with trisodium (–)-hydroxycitrate are better than those found with two out of the four isomers of chlorocitric acid, a compound that Roche touted vigorously as acting only via delay of gastric emptying.

One of the Roche inventors is Ann C. Sullivan, the same Roche researcher who on at least eight different occasions both before and after the dates of the issued patents maintained that HCA does not influence gastric emptying. Hence Guthrie, et al. persevered even in the face of

their own evidence in insisting the HCA's anorectic effects depended upon glucose receptors in the liver and not upon gastric emptying. However, in light to the examples given above, the present inventors feel that it is obvious that HCA does, in fact, substantially delay gastric emptying when used appropriately..

RESULTS

Treatment	Dose mmoles (mg)/kg	Stomach Contents		Serum	
		dpm $\times 10^3$ total per stomach	% of control	dpm $\times 10^3$ total per ml	% of control
Control	—	884 \pm 145 ^a	100	5.7 \pm 0.3	100
Trisodium Citrate	0.33 (97)	1180 \pm 208	133	6.4 \pm 0.1	112
Trisodium (—)-hydroxycitrate	0.33 (101)	1973 \pm 154***	223	4.5 \pm 0.3*	79
(+)-threo-Chlorocitric acid	0.33 (75)	1946 \pm 361*	220	3.4 \pm 0.6*	60
(—)-threo-Chlorocitric acid	0.33 (75)	2760 \pm 69***	312	1.9 \pm 0.4***	33
(—)-erythro-Chlorocitric acid	0.33 (75)	1491 \pm 232	169	5.1 \pm 0.4	90
(+)-erythro-Chlorocitric acid	0.33 (75)	1832 \pm 160**	207	5.0 \pm 0.4	88

^aEach value is the mean \pm S.E.

*P \leq 0.05

**P \leq 0.01

***P \leq 0.001

EXAMPLE 7

Fast Acting Capsule and Tablet Composition

All of the standard salts of HCA can be delivered after a fashion that rapidly increases exposure to the stomach lumen through the use of capsules or tablets containing sodium bicarbonate, potassium bicarbonate, magnesium carbonate or (less advantageously) calcium carbonate and similar compounds sufficient to cause the rapid release of the contents of the capsule or tablet when exposed to stomach contents. Hygroscopic salts of HCA, such as the potassium and sodium salts, will require initial processing with hydrophobic (but not acidophobic) coatings, etc. before being added to the capsules or tablets.

Example of a Fast-Releasing Formulation

Product	Mg/Capsule	%
1. Potassium-calcium HCA	200 mg	50.0
2. Sodium Bicarbonate	30 mg	7.50
3. Starch 1500	70 mg	17.50
4. Malic Acid	100 mg	25.00
TOTAL	400 mg	100.0%

The HCA salt is blended with starch 1500 and sodium bicarbonate; malic acid is then added and blended and the whole powdered material is passed through a #20 screen to allow even pouring and filling of capsules. If it is desired to make tablets out of this material, it would be mixed with 0.5% magnesium stearate and compressed on a rotary tablet machine. After entering the stomach the starch will initiate the immediate disintegration of the tablet or capsule and the sodium bicarbonate will mix with the malic acid to cause the rapid dispersal of the HCA. Numerous additional acids may be used to activate the bicarbonate, such as L-tartaric acid, citric acid, lactic acid, alginic acid, fumaric acid, aspartic acid and ascorbic acid. The formula can also omit the acid component and depend entirely upon the gastric acid of the stomach to induce the reaction with the bicarbonate.

EXAMPLE 8

Sustained Gastric Residence Composition

All of the standard salts of HCA can be delivered after a fashion that increases mean residence time in the stomach extended presentation to the stomach wall through the use capsules or tablets containing sodium bicarbonate or potassium bicarbonate plus alginic acid; also capsules or tablets containing sodium or potassium alginate. Hygroscopic salts of HCA, such as the potassium and sodium salts, will require initial processing with hydrophobic (but not acidophobic) coatings, etc. before being added to the capsules and tablets.

One means of increasing the residence time of HCA in the stomach is to use the simple formula in Example 7 and substitute alginic acid for malic acid. Sustaining the residence time of

the HCA in the stomach also can be accomplished by using an aqueous latex dispersion of ethyl cellulose known commercially as Surelease® or Aquatcoat®. This is sprayed onto the non- and moderately-hygroscopic HCA salts, such as the calcium and potassium-calcium salts, in a fluid bed dryer in a 0.5-1% coat. (Fully hygroscopic salts of HCA, such as the pure potassium and sodium salts, except under very dry conditions may first need to be pre-coated as described in the inventors' various patents and patents pending covering stable and controlled release of HCA.) The coated material is then admixed with alginic acid and sodium bicarbonate along with starch. The light water impermeable coat will dissolve from the HCA before being expelled from the stomach and some will be trapped in the foamy alginate bicarbonate material which will prolong it's dwell time in the stomach. Below is a capsule formulation of this product.

Sustained Residence Formulation		
Product	Mg/Capsule	Percent
1. Potassium-calcium HCA	400 mg	57.14%
2. Sodium Bicarbonate	50 mg	7.14%
3. Alginic Acid	200 mg	28.58%
4. Starch	50 mg	7.14%
TOTAL	700 mg	100.0%

The HCA is first sprayed with a latex dispersion of ethyl cellulose. When it is dry, it is blended with the remaining materials and placed through a #20 screen. When this is complete, the milled granulate is placed into capsules with a weight of 700 mg or compressed into tablets of similar weight. The disintegration rate should be 100% within 20 minutes.

EXAMPLE 9

Dry Packaged Meal Replacement Composition

It is feasible to supply HCA via dry packaged powders designed to be mixed with water or juice and consumed between meals or prior to meals. HCA mixed into tomato juice is especially successful, whereas HCA tends to bind to components in citrus, grape and many other juices.

Under normal commercial processing, sufficient moisture remains in food products to allow even HCA calcium salts to slowly bind to food components, such as tannins, gums, fibers and pectins. The much more active potassium and sodium salts of HCA are not practical unless they have undergone initial processing with hydrophobic coatings.

All of the commercial salts of HCA will bind to food components in dry mixtures if left in contact for any extended length of time. A lack of awareness of the fact that HCA salts must be prevented from being inactivated by food elements, phytonutrients, etc., has contributed greatly to failed and disappointing trials using the compound. Hence pretreatment of some sort is absolutely necessary.

Potassium-calcium HCA can be coated with a small dose of ethyl cellulose such as noted in example 8 and placed in a vacuum sealed envelope after being mixed with dried food and/or herb concentrates. The contents of the package later can be mixed with water and ingested 30 minutes to 1 hour before a regular meal or as a snack before bedtime. Capsaicin-based condiments and flavorings, such as pepper sauces, should be avoided in these snacks and meal replacements.

EXAMPLE 10

Compositions for Use in Liquids, Bars, Jelly-Like Products, Etc.

Because of the resulting non-gritty mouth feel, it is especially advantageous to pre-treat HCA salts with molten oils such as hydrogenated vegetable oil, glycerol monostearate, cetyl alcohol, stearyl alcohol and various high viscosity grades of conjugated polyethylene glycol, d- α -tocopheryl, polyethylene glycol succinate (TPGS) and similar compounds prior to being added to foodstuffs. Subsequent processing can allow the material, now rendered non-hygroscopic and non-reactive, to be further encased in gelatin, tapioca, gums/pectins, inulin, cellulose derivatives, etc., for inclusion in thick drinks, soft-center bars and candies, pudding snacks, jelly-like confections, “gummy” deliveries, liquid meal replacements, etc. Upon consumption, the HCA is released by mechanical means (chewing) and enters the stomach in conjunction with food and liquid. As such, the dosage of HCA can be taken via snacks or meal replacements and is accompanied by the items necessary to supply the volume that activates HCA-induced satiety.

EXAMPLE 11

Compounds for Additive and Synergistic Benefits

HCA may be used in conjunction with many agents that influence gastric emptying, such as citric acid, sodium or potassium citrate, other citric acid salts, sodium propionate, propionic acid, gallic acid, propyl gallate; extracts of marigold (*Calendula officinalis*); escins and other compounds from *Aesculus hippocastanum* seeds; extracts of the fruit of *Kochia scoparia*, and the roots and other parts of *Aralia elata*; saponins, especially Theasaponin E1 from the the seeds of the tea plant (*Camellia sinensis* L.); extracts from bay leaf (*Laurus nobilis*), especially costunolide and its active component, alpha-methylene-gamma-butyrolactone (*alpha*-MGBL); proteinase inhibitor extracts from potato and soybean sources; a variety of oleanolic acid glycosides from many sources; also herbal combinations such as one consisting of yerba mate, damiana and guarana.

Example of a Synergistic Fast-Releasing Formulation

Product	Mg/Capsule
1. Potassium-calcium HCA	200 mg
2. Sodium Bicarbonate	30 mg
3. Starch 1500	70 mg
4. Malic Acid	100 mg
5. Yerbe maté	112 mg
6. Guarana	95 mg
7. Damiana	36 mg
TOTAL	643 mg

The HCA salt is blended with starch 1500 and sodium bicarbonate; malic acid, yerbe maté, guarana and damiana are then added and blended and the whole powered material is passed through a #20 screen to allow even pouring and filling of capsules. If it is desired to make tablets out of this material, it would be mixed with 0.5% magnesium stearate and compressed on a rotary tablet machine. Three capsules are taken three times per day 30 to 60 minutes before meals with 8 - 16 ounces of apple, tomato or other juice; alternatively, 4 or 5 capsules are taken twice per day prior to lunch and supper.

CONCLUSIONS

(-)-Hydroxycitrate has a multitude of metabolic functions. The literature teaches that the compound reduces blood lipids, induces weight loss and decreases appetite in both animals and humans. However, the inventors have discovered that food and pharmaceutical compositions containing (-)-hydroxycitric acid, its salts, amides and esters can be employed for delaying gastric emptying and increasing receptive relaxation for preventing and treating diverse conditions. There are concomitant influences on glucagon-like peptides (GLP-1/2) and cholecystokinin (CCK). Altered gastric emptying and accommodation are found with forms of hypertension, liver dysfunction and gastrointestinal ulcers, especially duodenal ulcer. Numerous medications, such as antibiotics (erythromycin, indomethacin, etc.) and including even some diet drugs (e.g., Orlistat and other lipase inhibitors), can accelerate gastric emptying. Surgery, such as for peptic ulcers, itself can lead to clinical dumping syndrome, as can other types of surgery performed on the stomach. Other factors or conditions that lead to acceleration of gastric emptying include obesity, high-energy density of food, fat intolerance, early stages of noninsulin-dependent diabetes mellitus, Zollinger-Ellison syndrome, and intermittent episodes in other forms of diabetes. HCA delivered in the form of its potassium salt is efficacious at singly delivered dosages of between 150 mg and 5 grams, preferably at a dosage of between 500 mg and 3 grams for most individuals. Other salts, amides and esters are active at individual dosage ranges, with, for instance, the sodium salt acting similarly to the potassium salt whereas salts containing calcium are less active. Various delivery methods that preferentially expose HCA to stomach and duodenal receptors and sensors without undue binding of the compound to inactivating substances are provided. The safe and effective employment to delay gastric emptying is an entirely novel use of (-)-hydroxycitric acid, its derivatives and its salt forms.